

Use of Diphtheria Antitoxin (DAT) for Suspected Diphtheria Cases

IRB # 4167

BB IND 11184

Sponsored by:

**Centers for Disease Control and Prevention
Atlanta, GA**

February 2012

**Principal Investigator:
Tejpratap Tiwari, MD**

Co-Investigator

Thomas Clark, MD

**Meningitis and Vaccine-Preventable Diseases Branch
Division of Bacterial Diseases (MS C-25)
National Center for Immunization and Respiratory Diseases
Centers for Disease Control and Prevention
1600 Clifton Road NE
Atlanta, Georgia 30333**

TABLE OF CONTENTS

	<u>Page</u>
1.0 OBJECTIVE	3
2.0 BACKGROUND	3
3.0 PRODUCT INFORMATION	4
4.0 CLINICAL DESIGN	4
5.0 ELIGIBILITY	4
Therapeutic use	4
Prophylactic use	5
6.0 TREATMENT PROCEDURES	5
Precautionary measures	6
History suggesting increased risk from DAT administration	6
Desensitization	7
Administration	7
Possible adverse reactions following administration of DAT	8
7.0 LABORATORY TESTING	9
8.0 LOCAL AND STATE HEALTH DEPARTMENT NOTIFICATION	9
9.0 DATA COLLECTION AND STORAGE	10
10.0 REFERENCES	12

Appendix I: Patient Informed Consent

Appendix II: Diphtheria Antitoxin Treatment and Adverse Effects Form

Appendix III: CDC Diphtheria Worksheet

Appendix IV: Information for Close Contacts

1.0 OBJECTIVE

The purpose of this IND is to provide a treatment plan for the use of an unlicensed diphtheria antitoxin (DAT) for treatment of acute cases of diphtheria and, under exceptional circumstances, to provide passive, transient protection against diphtheria in an exposed contact.

No licensed diphtheria antitoxin is available in the United States.

2.0 BACKGROUND

Diphtheria is a clinical syndrome caused by a polypeptide exotoxin produced by the bacterial pathogen *Corynebacterium diphtheriae*; non-toxin-producing strains of *C. diphtheriae* are not associated with the syndrome but can cause localized inflammation. The severe local and systemic manifestations of diphtheria result after diphtheria toxin binds to a wide range of mammalian cells, including epithelial, nerve and muscle cells. The toxin interferes with enzymes necessary for protein synthesis, leading to cell damage and death. Local effects include severe inflammation and pseudomembrane (a firmly adherent exudate that looks like a membrane) formation in the pharynx or larynx, which can progress to airway obstruction. Systemic effects include myocarditis, polyneuritis, and, rarely, renal failure.

There are four biotypes of *C. diphtheriae*: *gravis*, *mitis*, *belfanti* and *intermedius*. All four biotypes are capable of producing an identical exotoxin. No difference in pathogenicity has been demonstrated among the three biotypes. Rare human cases of infection with toxin-producing *Corynebacterium ulcerans* cause identical clinical outcomes (1). Severe clinical manifestations result from absorption of toxin (either local or systemic) produced by infection with *C. diphtheriae* at an epithelial site (usually pharynx, occasionally nasal lining, or skin). The onset of disease is insidious. Following an incubation period of 1-5 days, low-grade fever begins and a pharyngeal pseudomembrane develops over 2-3 days, along with lymphadenopathy and diffuse systemic toxicity, resulting in a rapid, thready pulse, weakness, and irritability. Although the systemic effects of diphtheria can occur in the first week of illness, they usually occur later (1-2 weeks after onset for myocarditis, 2-8 weeks for neuritis).

The hallmark of suspected diphtheria is a febrile, membranous pharyngitis of insidious onset. In a minority of instances, diphtheria can result from an isolated diphtherial infection in the larynx, nasal lining, or skin. Other diseases that can occasionally produce a similar membranous pharyngitis include streptococcal pharyngitis and infectious mononucleosis. Patients who have been treated with immunosuppressive drugs can present with a membrane that mimics diphtheria. Isolated diphtherial laryngitis can usually be differentiated from *Haemophilus influenzae* type b epiglottitis, spasmodic croup, or the presence of a foreign body by the gradual onset of diphtherial disease. Differentiation of isolated diphtherial laryngitis from viral laryngotracheitis or bacterial tracheitis can be difficult on the basis of symptoms alone.

Diphtheria antitoxin (DAT) was first produced in the 1890s and is still produced using serum from horses hyperimmunized with diphtheria toxoid. The evidence for efficacy of DAT is based on observations and studies done several decades ago. Mortality rates for clinical diphtheria frequently exceeded 50% in the pre-antitoxin era. Almost as soon as antitoxin was available, clinical experience showed dramatic declines in mortality in groups of patients treated with antitoxin compared to historical control groups or groups treated at hospitals not using antitoxin. In one controlled trial in which patients at a hospital were allocated to antitoxin treatment or no antitoxin treatment on an alternating day schedule, mortality in treated patients was 3.3% compared to 12.2% in untreated patients (2). It was also shown that early treatment is critical, with the degree of protection from DAT inversely related to the duration of clinical illness preceding its administration. Mortality increased progressively within the interval from onset of illness to treatment, with a sharp increase from 4% mortality in those treated with antitoxin within 24-48 hours to 16.1% in those

treated on the third day of illness (3). Mortality rates continued to increase with more prolonged intervals, reaching 29.9% in those treated 7 or more days after onset. Current thinking is that toxin fixes to susceptible cells early in disease and fixed toxin is not neutralized by antitoxin (4).

The management of a patient with suspected diphtheria includes:

- (i) Administration of diphtheria antitoxin as soon as possible after testing for hypersensitivity to horse serum; early administration of appropriate antitoxin is critical for survival (5).
- (ii) Establishing the diagnosis through appropriate bacterial cultures;
- (iii) Administration of antibiotics; and
- (iv) Appropriate supportive care including special attention to maintaining an adequate airway in the presence of laryngeal or extensive pharyngeal membranes and to careful monitoring for cardiac rhythm disturbances or other manifestations of myocarditis.

Diphtheria is currently a rare disease in the United States; 0-5 cases a year have been reported annually since 1990. There is evidence that toxigenic strains of diphtheria continue to circulate in at least limited areas of the United States (6). In 2003, a case of respiratory diphtheria was imported from a country that is endemic for the disease. Access to diphtheria antitoxin is essential to ensure effective treatment of all cases of diphtheria.

3.0 PRODUCT INFORMATION

A U.S. licensed DAT product manufactured by Connaught was available until 1996 after which it was no longer manufactured in the U.S. In 1997, a DAT product manufactured by Pasteur Merieux and licensed in France and other European countries, was made available in the U.S. through an IND protocol (BB IND 6937). Pasteur Merieux stopped manufacturing DAT in 2002. CDC has been unable to identify a manufacturer that plans to license a DAT product in the U.S. and thus has sought alternative, international sources. A DAT product manufactured by the Instituto Butantan in São Paulo, Brazil is available for use in the United States under this IND protocol. This product is similar to the previously licensed DAT.

4.0 CLINICAL DESIGN

This is a protocol to allow DAT treatment for patients with suspected diphtheria infection. Physicians requesting diphtheria antitoxin should contact the CDC's Emergency Operations Center (EOC) at 770-488-7100. Decisions to release DAT will be made by the CDC diphtheria duty officer or his/her designee in discussion with the treating physician. The decision to administer DAT (once dispensed) to a patient will be made by the treating physician.

5.0 ELIGIBILITY

Therapeutic use

Patients who have probable or confirmed respiratory diphtheria are eligible to receive DAT. The Council of State and Territorial Epidemiologists approved of the following case definition for clinical respiratory diphtheria: an upper respiratory tract illness characterized by sore throat, a low grade fever, and an adherent membrane of the tonsil(s), pharynx, larynx, and/or nose. A confirmed case is either a clinical case from which *C. diphtheria* is isolated from respiratory specimens (nasal or throat swab, membrane tissue) or a clinical case that is epidemiologically linked to a laboratory- confirmed diphtheria case. A probable case is a clinically compatible case that is not laboratory confirmed and is not epidemiologically linked to a laboratory confirmed case.

A patient's eligibility for treatment will be determined through discussion between the CDC diphtheria duty officer and the treating physician. The diphtheria duty officer will always release DAT if, following the discussion, it is still the decision of the treating physician to request and administer DAT. The final decision to administer DAT to a case-patient lies with the treating physician. The treating physician may decide against using DAT after it is released.

DAT should be released and administered without delay to:

- A. All cases of respiratory diphtheria with laboratory-confirmed toxigenic *Corynebacterium diphtheriae*.
- B. Probable cases. Diphtheria should be strongly suspected in a probable case-patient who is toxic in appearance and
 - is without another clearly established diagnosis and/or
 - has rapidly worsening illness and/or
 - has history of recent travel to a country where diphtheria is endemic or epidemic and/or
 - was exposed to travelers from countries with endemic or epidemic diphtheria and/or
 - has history of recent contact with dairy animals and/or
 - was never vaccinated or is not up-to-date with diphtheria toxoid vaccination

For probable cases which are considered to have a low probability for diphtheria, the duty officer will encourage the requesting physician to aggressively consider other diagnoses. However the final decision to request and administer DAT to a patient lies with the requesting physician.

C. Case-patients who have isolated or localized lesions in the nose, eye, skin, or other anatomical sites from which is obtained, and in whom there are signs of systemic toxicity (fever, tachycardia, and weakness). DAT treatment is not routinely indicated for treatment of diphtheria skin infection; toxigenic sequelae are rare when the infection is limited to the skin and when the case-patient is up to date on vaccination with diphtheria toxoid.

Prophylactic use

DAT is used prophylactically only under exceptional circumstances. Eligibility for prophylactic use of DAT will be limited to the following situations:

- An individual who has had known exposure to toxigenic *C. diphtheriae* (or possibly other toxigenic *Corynebacteria*).
- An individual who is not up-to-date for vaccination against diphtheria and who has suspected or known exposure to toxigenic *Corynebacteria*.
- An individual who cannot be kept under surveillance for the development of clinical symptoms or is not available for follow-up of results of culturing for the diphtheria organism after exposure to a known or suspected infection with toxigenic *Corynebacteria*.

Each request for use of DAT for prophylactic use will require detailed discussion of all possible options with the diphtheria duty officer.

Under the rare circumstances when these conditions are met, the recommended dose of DAT is 10,000 units (after appropriate sensitivity testing). These patients also should be given prophylactic antibiotics and appropriate up-date vaccination with diphtheria toxoid.

6.0 TREATMENT PROCEDURES

DAT is an equine serum product and precautionary measures are recommended (described below) for all

patients. A history of hypersensitivity reactions to equine proteins or positive skin testing in a patient indicates that additional special precautions should be taken in giving DAT or other equine material to these patients.

Precautionary measures

All patients should have the following:

- An appropriate history taken for factors suggesting increased risk (see below)
- Sensitivity testing to DAT*
- Careful monitoring during sensitivity testing and DAT administration for evidence of hypotension and bronchoconstriction

Patients with a history suggesting increased risk should have the following:

- Initial sensitivity testing with a reduced dose*
- Very careful monitoring during sensitivity testing and DAT administration for evidence of hypotension and bronchoconstriction

*Patients with positive sensitivity testing to DAT or with a history of hypersensitivity reactions to equine proteins (even with a negative or equivocal sensitivity test) should have “desensitization” performed (see below).

Personnel who test for sensitivity to or administer DAT should be trained to treat anaphylactic reactions. The necessary medications, equipment, and staff competent to maintain the patency of the airway and to treat cardiovascular collapse must be immediately available.

History suggesting increased risk from DAT administration

Patients with the following history may be at increased risk of developing serious anaphylactic reactions upon receipt of equine origin serum administered subcutaneously, intramuscularly, or intravenously:

- Asthma, allergic rhinitis, or urticaria
- Asthma, allergic rhinitis, or urticaria or other symptoms of distress when in proximity to horses
- Previous injection of serum of equine origin

These patients must be desensitized (see below) before administering DAT even with a negative or equivocal sensitivity testing.

Tests for Sensitivity to DAT

A test for sensitivity to DAT should be carried out each time DAT is administered. Sensitivity to DAT may be assessed by two methods (7): the scratch, prick, or puncture skin test is followed by an intradermal test if the skin test is negative. This order is recommended as the skin test is thought to be safe while the intradermal test has been reported to cause fatal anaphylactic reactions. Personnel who perform skin tests for sensitivity to DAT should be trained to recognize and treat acute anaphylaxis. The necessary medications, equipment, and staff competent to maintain the patency of the airway and to treat cardiovascular collapse must be immediately available.

A. Scratch, prick, or puncture skin test

After cleaning a skin site on the volar surface of the patient’s forearm with alcohol and air drying, make a superficial scratch, prick, or puncture using a sterile needle or other sterile sharp instrument, breaking the skin but not drawing blood. Apply one drop of a 1:100 dilution of the serum in normal saline to the site. Positive (histamine) and negative (physiologic saline) control tests should also be applied to similar scratch, prick, or puncture sites. A positive scratch test is a wheal with surrounding erythema at least 3 mm larger than the negative control test, read at 15-20 minutes. The histamine

control must be positive for valid interpretation; a positive response consists of a wheal at the scratch site surrounded by an erythematous area. If the scratch test is negative, an intradermal (ID) test is performed.

B. Intradermal (ID) test

Administer a dose of 0.02 ml of 1:1,000 saline-diluted serum intradermally; this quantity should raise a small ID wheal. If the test is negative, repeat it using a 1:100 dilution. In persons with a negative history for animal allergy and negative history for prior exposure to animal serum, the stronger (1:100) dilution of serum may be used in the ID test without prior testing with the lower dilution (1:1,000). Positive (histamine) and negative (physiologic saline) intradermal control tests should be applied. Interpretation of the ID test is done as with the scratch test.

A positive skin test indicates the probability of sensitivity with some correlation between the severity of the reaction on skin testing and the likelihood and severity of reaction to the DAT. However, a negative skin test does not preclude the possibility of an adverse reaction. In addition, antihistamines (and possibly other drugs such as tricyclic antidepressants) administered previously can interfere with the results of skin testing for periods of one day or longer depending on the antihistamine. DAT should be administered cautiously even in patients with negative skin tests.

Desensitization

Patients with positive sensitivity testing to DAT or with a history of hypersensitivity reactions to equine proteins (even with a negative or equivocal sensitivity test) should undergo *desensitization*. Tables 1 & 2 (appended) serve as guides for desensitization. Table 1 gives an intravenous (IV) regimen and Table 2 gives an intramuscular (IM) regimen (7). The IV route is considered safer because it offers better control.

The personnel performing desensitization need to have the expertise to treat anaphylaxis and the necessary equipment and medications available. Some physicians recommend concurrent treatment with an oral or IM antihistamine with or without IV administration of a corticosteroid such as hydrocortisone or methylprednisolone. **The protection from anaphylaxis afforded by giving DAT according to the desensitization treatment schedule requires that no interruption occur in the sequence of administration of doses; if an interruption occurs the protection is lost.**

Administration

Route:

The intravenous (IV) route is the preferred route of administration, especially in severe cases. The antitoxin should be mixed in 250–500 mL of normal saline and administered over 2–4 hours. The antitoxin may be given intramuscularly (IM) in mild or moderate cases.

Temperature

Antitoxin should be warmed to 32–34°C (90–95°F) before injection. Warming above the recommended temperature should be carefully avoided because the DAT proteins will denature.

Dosage

- A. Perform sensitivity tests, and desensitization if necessary.
- B. Give the entire treatment dose of antitoxin intravenously (or intramuscularly) in a single administration (except for series of injections needed for desensitization). When using the intravenous route, the antitoxin should be diluted in physiologic saline and administered slowly over several hours, closely monitoring for anaphylaxis.
- C. The recommended DAT treatment dosage ranges (8) are:

- Pharyngeal or laryngeal disease of 2 days duration: 20,000 to 40,000 units
 - Nasopharyngeal disease: 40,000 to 60,000 units
 - Systemic disease of 3 or more days' duration, or any patient with diffuse swelling of the neck: 80,000 to 100,000 units
 - Skin lesions only: 20,000 to 40,000 (for cases where treatment is indicated)
- D. Give children the same dose as adults.
- E. Repeated doses of DAT after an appropriate initial dose are not recommended and may increase the risk of adverse reaction.
- F. Appropriate antimicrobial agents in full therapeutic dosages should be started.

Any person with clinical symptoms of diphtheria should receive DAT as soon as it can be made available, without waiting for bacteriologic confirmation of the diagnosis. Supportive treatment should be continued until all local and general symptoms are controlled.

Prophylactic regimen

Patients and contacts should be reported urgently to local and/or state health department personnel. All asymptomatic, unimmunized contacts of patients with diphtheria should receive prophylactic antimicrobial therapy after specimens for cultures are obtained. Specimens for culture should be obtained again after treatment. Immunization with diphtheria toxoid should be given, if necessary, and surveillance for illness continued for seven days. This is the standard of care for contacts of a diphtheria case.

Prophylactic treatment with DAT can be considered but is recommended only in exceptional circumstances. For example, a contact who has had known exposure to a case and would not have access to medical personnel (unavoidable travel to a developing country) might be considered for DAT. Such exceptional circumstances should be discussed in detail with the CDC diphtheria epidemiologist on duty. If it is concluded that a close contact may benefit from receiving DAT, DAT can be administered in addition to antimicrobial prophylaxis and immunization with diphtheria toxoid. Before administering DAT:

- A. Perform appropriate sensitivity tests.
- B. If sensitivity testing is negative, give 10,000 units intramuscularly. The dose depends on the length of time since exposure, the extent of the exposure and the medical condition of the individual.
- C. If sensitivity testing is positive, proceed with desensitization schedule outlined above.

Possible adverse reactions following administration of DAT

Anaphylactic Reaction: Although onset and severity are highly variable, anaphylaxis usually begins in susceptible patients within minutes after exposure to DAT; in general the more rapid the onset, the more severe the reaction. The major manifestations are 1) cutaneous, including pruritus, flushing, urticaria, and angioedema; 2) respiratory, including hoarse voice and stridor, wheeze, dyspnea, and cyanosis; and 3) cardiovascular, including a rapid, weak pulse, hypotension, and arrhythmias. Anaphylaxis is a major medical emergency.

In the event of an anaphylactic reaction, treatment will depend on the nature and severity of the reaction. Parenterally administered epinephrine is the primary drug for all types of reactions. Antihistamines should also be given. Additional medications, depending on the severity of the reaction may include corticosteroids, alpha-adrenergic blocking agents, aminophylline, and beta-2 agonists (7).

Febrile Reaction:

When fever occurs, it usually develops twenty minutes to one hour after the injection of serum or antitoxin.

It is characterized by a chilly sensation, slight dyspnea and a rapid rise in temperature. Most febrile reactions are mild and can be treated with antipyretics alone; severe reactions may require other measures (tepid water baths, etc) to reduce the temperature.

Serum Sickness:

The symptoms of serum sickness are fever, maculopapular skin rashes, or urticaria in milder forms (90% of instances) with arthritis, arthralgia, and lymphadenopathy also possible in more severe forms. Rarely, angioedema, glomerulonephritis, Guillain-Barré syndrome, peripheral neuritis, or myocarditis can occur. The onset of symptoms is usually 7 to 10 days (range 5 –24 days) after initial exposure to the foreign protein (DAT). Onset can be as short as several hours to 3 days after re-administration of serum in persons who have received a dose of animal serum in the past; these individuals are also more likely to develop serum sickness. Mild cases of serum sickness frequently resolve spontaneously over a few days to 2 weeks. Medications that may be helpful include antihistamines, non-steroidal anti-inflammatory drugs, and corticosteroids (7).

Febrile reactions and serum sickness are not IgE mediated and therefore are not predicted by skin testing. The frequency of anaphylaxis and serum sickness is partially dependent on the frequency of previous administration of animal serums in the population; this frequency is now low. Recent data on the frequency of adverse reactions to horse serum products is extremely limited due to their infrequent use. In a review of 1,433 diphtheria cases treated with antitoxin between 1940 and 1950, the frequency of adverse reactions was as follows: anaphylaxis, 0.6% (without any fatalities); febrile reactions, 4.0%; and serum sickness, 8.8% (6).

7.0 LABORATORY TESTING

Although the decision to administer DAT in a suspected case of diphtheria must frequently be made in the absence of confirmatory laboratory evidence, it is essential to obtain the specimens to confirm the diagnosis early in the course of illness, and if possible, before the administration of antibiotics. The recommended specimens include the following:

- A. Throat, membrane (swabs or fragments), and nasal swab specimens for diphtheria culture. These are plated on special media. The laboratory must be notified to look for *C. diphtheriae* so that the appropriate, special medium is available, and laboratory personnel must have the necessary expertise to process cultures for *C. diphtheriae*. In patients who have not yet had specimens collected for culture, or who have had cultures processed that were negative for *C. diphtheriae*, the diphtheria duty officer at CDC may recommend that existing or new specimens be sent directly to the CDC Diphtheria Laboratory for culture and polymerase chain reaction (PCR) testing. Testing by PCR has the capability of identifying *C. diphtheriae* toxin from swabs or from membrane specimens when cultures are negative. Tissue and swabs for culture and PCR should be maintained in transport medium or in a sterile container kept moist with sterile, non-bacterial static saline until they reach the laboratory. Specimens for culture and PCR *should not be placed in formaldehyde*.

All *Corynebacterium* strains isolated and suspected of being toxin producers should be forwarded to the CDC Diphtheria Laboratory (telephone 404 639-1231) for confirmation and toxigenicity testing.

- B. A serum specimen can be obtained from the patient before antitoxin administration and tested for diphtheria antibodies. A low titer level (<0.01 IU/ml) indicates susceptibility but does not confirm the diagnosis. A high titer (>0.1 IU/ml) suggests that a diagnosis is less likely to be diphtheria.

8.0 LOCAL AND STATE HEALTH DEPARTMENT NOTIFICATION

It is a requirement that cases of suspected diphtheria (e.g., cases for whom DAT is requested) be reported to local and state health departments. A physician who requests and administers DAT will notify the local and state health departments. The CDC duty officer will also notify the state health department of any DAT that is released. Public health officials will assist in identifying contacts at risk of infection, will often obtain cultures, and will facilitate antibiotic prophylaxis of contacts when necessary to prevent spread to other members of the community.

9.0 DATA COLLECTION AND STORAGE

A requirement for release of DAT under IND is that CDC will obtain and store information relating to the patient's illness (including patient identifiers). Data obtained will be shared with the state health departments for public health investigation. In the event of serious adverse reactions, complete medical records may be requested for evaluation by the principal investigator from the treating physician. Serious adverse reactions will be reported to CDC IRB and FDA. Stored data will be analyzed as needed by CDC staff to prepare annual summary reports for renewal or continuation of the protocol with the CDC IRB and FDA. The data containing personal identifiers will be stored in locked file cabinets. This information will be kept strictly confidential. All forms should be returned directly to the CDC Drug Service.

CDC Drug Service, Mailstop D-09
1600 Clifton Rd.
Atlanta, GA 30306
Phone: 404-639-3670
Fax: 404-639-3717

Table 1. Desensitization to DAT - Intravenous route (7)

Dose number *	Dilution of DAT in normal saline	Amount of injection (cc)
1	1:1,000	0.1
2	1:1,000	0.3
3	1:1,000	0.6
4	1:100	0.1
5	1:100	0.3
6	1:100	0.6
7	1:10	0.1
8	1:10	0.3
9	1:10	0.6
10	undiluted	0.1
11	undiluted	0.2
12	undiluted	0.6
13	undiluted	1.0

* Administer at 15 minute intervals.

Table 2. Desensitization to DAT - Intradermal, subcutaneous and intramuscular route (7)

Dose number *	Route of administration	Dilution of DAT in normal saline	Amount of injection (cc)
1	ID	1:1,000	0.1
2	ID	1:1,000	0.3
3	SC	1:1,000	0.6
4	SC	1:100	0.1
5	SC	1:100	0.3
6	SC	1:100	0.6
7	SC	1:10	0.1
8	SC	1:10	0.3
9	SC	1:10	0.6
10	SC	undiluted	0.1
11	SC	undiluted	0.2
12	IM	undiluted	0.6
13	IM	undiluted	1.0

* Administer at 15-minute intervals.

10.0 REFERENCES

1. Centers for Disease Control. Respiratory Diphtheria caused by *Corynebacterium ulcerans*: Terre-Haute, Indiana, 1996. MMWR 1997;46:330 –2.
2. Diphtheria; other corynebacterial and coryneform infections. In Topley and Wilson's principles of bacteriology, virology and immunity (Chapter 60). 5th ed. Baltimore, MD: Williams and Wilkins, 1964:1695 –98.
3. Naiditch MJ, Bower AG. Diphtheria. A study of 1433 cases observed during a ten year period at the Los Angeles County Hospital. Am J Med. 1954;17:229-45
4. Placido Sousa C, Evans DG. British Journal of Experimental Pathology. 1957;38:644-.
5. Farizo KM, Strebel PM, Chen RT, et al. Fatal respiratory disease due to *Corynebacterium diphtheriae*: case report and review of guidelines for management, investigation, and control. Clinical Infectious Diseases 1993;16:59-68.
6. CDC. Unpublished data.
7. American Academy of Pediatrics. Active and Passive immunization. In: Pickering LK, ed. 2000 Red Book: Report of the Committee on Infectious Diseases, 25th ed. Elk Grove Village, IL: American Academy of Pediatrics;2000:47-51
8. American Academy of Pediatrics. Diphtheria. In: Pickering LK, ed. 2000 Red Book: Report of the Committee on Infectious Diseases, 25th ed. Elk Grove Village, IL: American Academy of Pediatrics;2000:230-34

This document can be found on the CDC website at:
<http://www.cdc.gov/diphtheria/downloads/protocol.pdf>